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SPAWNING PERIODICITY OF SEA URCHINS AT SETO

II. *DIADEMA SETOSUM*[1]

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With 2 Text-figures

Diadema setosum (LESKE) is a kind of sea urchins widely distributed over the tropical and subtropical parts of the Indo-Pacific, its locality reaching northerly the southern coasts of Japan. In the foregoing paper of this series the first author (KOBAYASHI, 1967) insisted that *Mespilia globulus* manifested a hemi-lunar periodicity with regard to the spawning during its breeding season extending from July to August, and suggested that such periodicity might be found commonly in some other sea urchins at Seto. According to FOX (1924 a) *Diadema setosum* at Suez spawns at each full moon during the breeding season and reproduces again a fresh stock of genital products to be spawned at respective ensuing full moon. MORTENSEN (1938) admitted FOX's observations of this periodicity as a rare exceptional phenomenon at Suez. On the other hand, RANDALL, SCHROEDER and STARCK (1964) seemed to be of the opinion that no lunar periodicity of reproduction of *Diadema antillarum* was found in the West Indian Region. Furthermore, KĚCKĚS (1966) reported that no correlation was found between the moon cycle and the maturity of *Paracentrotus lividus* and *Sphaerechinus granularis* in the North Adriatic. In Japan, YOSHIDA (1952) produced by histological examination of the gonad an evidence reconcilable with FOX's view and KUME and DAN (1957) suggest that *Diadema setosum* spawns at the night of full moon at Misaki. To approach the problem whether the spawning of *Diadema setosum* is related to the lunar cycle or it is of a hemi-lunar periodicity, changes in the gonad volume and the germ cell maturity were examined through several days around the new and full moon in July and August, 1966.

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Material and Method

At Seto, *Diadema setosum* is abundant in rocky shore surrounding the Seto Marine Biological Laboratory, being densely crowded below the low water mark during the breeding season. The sea urchins were collected from the shallow water at three different places enough apart from one another, shortly before the water is lowest. The specimens used for observations were all mature individuals with the test volume larger than 18.5 cc, over 110 cc at the maximum in both sexes. Daily observations were continued for three periods respectively from the 16th to the 21st of July, from the 29th of July to the 3rd of August, and from the 16th to the 18th of August; throughout these periods the new moon was on the 19th of July and on the 16th of August, and the full moon on the 2nd of August.

Table 1a. Classification of maturation grades in female.

grade of ripening	GT ratio	color of gonad	texture of gonad tissue	gamete in smear	state of gonad
I	<9.9%	dark brownish yellow	very hard	empty	wholly spent
II	10.0–19.9%	brownish yellow	coarse	almost empty	spent
III	20.0–29.9%	yellow	soft	ova in various stage of ripening	in ripening
IV	30.0–39.9%	light yellow	softer than III	ova mostly ripen	mature
V	40.0%<	same, transparent	very soft	full of ripe ovum	fully matured

Table 1b. Classification of maturation grades in male.

grade of ripening	GT ratio	color of gonad	texture of gonad tissue	gamete in smear	state of gonad
I	<9.9%	dark brownish yellow	very hard	empty	wholly spent
II	10.0–19.9%	brownish yellow	coarse	almost empty	spent
III	20.0–29.9%	yellow	soft	ripe sperms	ripening
IV	30.0%<	light yellow	softer than III	full of ripe sperms	matured

As to respective specimens, the gonad volume was measured and fresh smear preparations of squashed gonad were examined under microscope. As shown in Table 1, the maturation grade of the gonad was divided into five classes in female and four in male, according to the ratio of the gonad volume to the test volume or the GT ratio. This classification is roughly correlated with the differences of the gonad in color and texture of the tissue. The gonadal contents in each maturation grade were quite similar to those described in *Mespilia* (KOBAYASHI, 1967). In the preliminary tests, the GT ratio after a forced spawning with KCl was examined. It was interesting to find that the GT ratio was 15% at the new moon in July and slightly less than 5% at the ensuing full moon; the maturation of the gonad was respectively in grade II and in I. There was no noticeable difference between the

sexes in the maturity. These data mentioned above seem to show that the sea urchins did not become wholly spent after a forced spawning at the new moon, while they became wholly spent at the full moon.

Results

I. The first period

The period covered six days from July 16 to 21, the new moon being on the 19th. The results of observations made in this period are summarized in Table 2a and the occurrences of the female and male in respective maturation grades in percent are illustrated in Fig. 1a, b. None of the wholly spent female of grade I was found during this period; this can not be accidental, because the females just after the forced spawning with KCl at the new moon in this period were found in grade II but not in the state of wholly spent. The occurrence of mature female in grade IV and V was remarkably high on the first day and in the three days before the new moon, but as shown by the solid line in Fig. 1a it decreased with days to the minimum on the day after the new moon and then increased again next day. Spent females in grade II were not found on the first day but appeared next day. The occurrence of the spent female exhibited a striking contrast against that of the mature female, which showed a mode on the day of new moon as shown by the broken line in the figure. The succession found in the occurrences of the female in respective grades is seemingly very suggestive of the maturation rate of the female during this period. On the first day, 25% of females were in grade III, while the remaining in grade IV; and there was no spent individual. On the second day, about 33% of females were spent, while 67% of them were fully mature and in grade V; but there was no female in grade III or IV. These facts seem to suggest that some of the females in grade IV on the first day had spawned and the remaining females in earlier stages of grade IV, together with the females in grade III, were advanced to grade V within 24 hours. On the third day or the day before the new moon, the females of grade III, up to 37.5%, appeared again, while the females in grade II and V were 37.5% and 25% respectively; but there was no female in grade IV. This amply suggests that nearly one half of fully mature females on the second day became spent on the third day accomplishing the spawning. On the day of new moon the occurrence of the spent female was remarkably increased to 83%, while the female in grade IV was 17%; there was no female in grade III or V. This shows that a half of females in grade III on the day before became mature on the day of new moon, while females in late grade III or more advanced stages had accomplished the spawning and were already in grade II on that day. On the day next the new moon the spent females decreased very slightly and 20% of females were found in grade III. Disappearance of the mature female and appearance of the female in grade III indicate that mature females on the day of new moon became spent next day, while some of spent females

Table 2a. Records of examined individuals in the first period of observation.

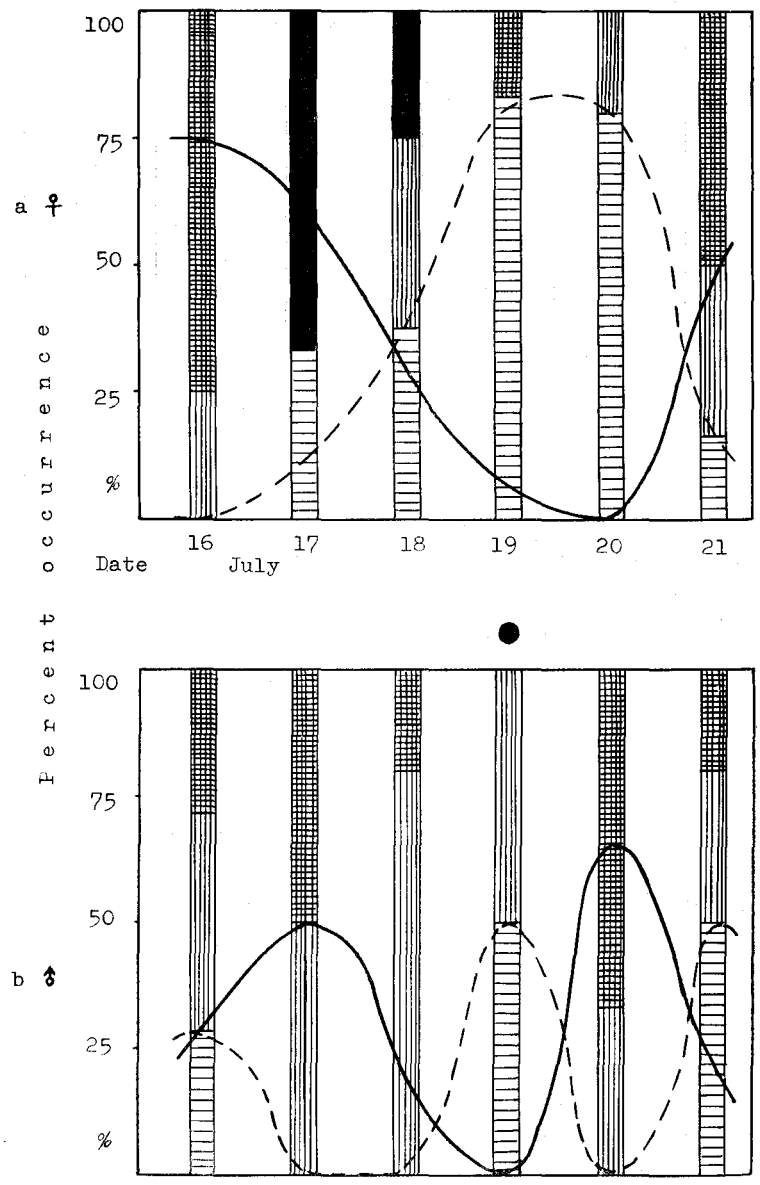
date \ sex	female			male		
	test volume (cc)	GT ratio	maturity grade	test volume (cc)	GT ratio	maturity grade
16/VII	19.5	25.6	III	50.0	37.0	IV
	35.0	35.7	IV	31.5	23.8	III
	74.0	31.8	IV	37.0	18.4	II
	71.5	37.1	IV	22.0	25.0	III
				18.5	18.9	II
				27.5	32.7	IV
				22.5	24.4	III
17/VII	28.5	19.3	II	41.0	23.2	III
	37.0	45.9	V	51.0	22.5	III
	57.5	48.7	V	71.0	27.5	III
				37.5	32.0	IV
				50.0	25.0	III
				26.5	32.1	IV
				50.0	35.0	IV
				25.0	30.0	IV
18/VII	28.5	40.4	V	39.5	24.1	III
	71.5	20.3	III	50.0	27.0	III
	50.0	40.0	V	68.5	23.4	III
	34.0	17.6	II	54.0	38.9	IV
	56.0	15.2	II	43.0	26.7	III
	70.0	18.6	II			
	27.0	29.6	III			
	35.0	21.4	III			
19/VII	40.5	18.5	II	91.5	19.1	II
	77.0	13.6	II	67.5	18.5	II
	45.5	18.7	II	57.0	19.3	II
	45.0	34.4	IV	29.5	18.6	II
	34.5	13.0	II	30.0	20.0	III
	42.5	17.6	II	44.5	27.0	III
				30.5	21.3	III
				43.5	28.7	III
				30.5	19.7	II
				57.5	20.0	III
20/VII	44.5	24.5	III	69.5	24.5	III
	68.0	16.9	II	69.5	30.9	IV
	75.0	16.7	II	78.0	36.5	IV
	35.0	17.0	II	93.0	31.2	IV
	63.5	17.3	II	69.5	36.0	IV
				67.5	23.0	III

Table 2a. (continued)

sex / date	female			male		
	test volume (cc)	GT ratio	maturity grade	test volume (cc)	GT ratio	maturity grade
21/VII	48.5	22.7	III	58.5	12.0	II
	39.5	24.3	III	27.5	30.0	IV
	38.5	19.5	II	38.0	11.8	II
	43.0	37.2	IV	47.5	23.2	III
	30.0	38.3	IV	51.0	29.4	III
	33.5	34.3	IV	32.0	17.2	II

advanced to grade III. On the last day of the period, namely two days after the new moon, the proportion of respective grades turned suddenly; mature females in grade IV attained to 50%, females in grade III increased to 34%, while the spent female decreased to 16%. It is surmised that the mature females on that day were derived from the females in grade III and some of the spent individuals in late grade II on the day before. By checking these data, the time duration needed for the maturation of spent females may be roughly estimated. About a half of mature females on the first day became spent next day and then at least one half of those spent females were found matured on the day of new moon or the 4th day of the period, but the other half must have been fully mature on the 3rd day and accomplished the spawning already on the day of new moon. Thus it is supposed that two or three days were needed for the maturation of a batch of germ cells remained in the ovary after the spawning.

In the male the fluctuation of the occurrence of respective grades of maturity was not so simple as in the female, although the complete absence of the wholly spent in grade I was common in both sexes during this period. On the first day of the period the spent male in grade II occupied nearly 29% of the whole males examined. As shown by the broken line in Fig. 1b, the frequency curve of the spent male shows two modes as high as 50%, one on the day of new moon and the other two days later. Between these maxima, however, the spent male was completely absent. In contrast to the spent, the mature male attained the first maximum on the second day and the second on the day next the new moon, respectively 50% and 67%. It is to be noticed that the mature males on the 17th or two days before the new moon were as much as 50% but dropped to 20% next day, nevertheless no spent was found on the 18th. A tentative but very probable explanation of this phenomenon may be that on the 17th about a half of mature males shed sperms incompletely so that they remained in grade III next day.



Text-figure 1. Occurrences of individuals of respective maturation grades in percent in the period from July 16 to 21, 1966, with the new moon on the 19th.

●: new moon, ○: full moon, —: successive changes of mature or fully mature, ···: successive changes of spent or wholly spent

■ : fully mature (V), ▦ : mature (IV), ▥ : in ripening (III)

▤ : spent (II), □ : wholly spent (I)

II. The second period

The second period covered six days from July 29 to August 3; the full moon was on August 2. The results of observations are summarized in Table 2b. The striking feature in the sexual maturation in this period was the appearance of wholly spent individuals in both sexes. This was quite consistent with the result of the forced spawning mode on the day of full moon. After the treatment, mature individuals of both sexes became wholly spent and the GT ratio dropped below 5%.

In the female, there was no spent on the first day of the period; spent females appeared on the second day and reached the first maximum on the third day when wholly spent ones occupied 50% of the examined specimens. Then, on the day of full moon, all the examined specimens were spent and 50% of them were wholly spent. Next day spent females decreased a little, as females in grade III appeared. As seen in Fig. 2a the occurrence fluctuation of mature females inclusive of grade IV and V shown by a solid line assumes exactly a mirror image of the fluctuation of spent individuals. On the first day, mature individuals were about 72% of all examined specimens and 28% of them were full mature. Full mature individuals disappeared next day, and on the third day no mature individual was detected. A small number of mature individuals appeared on the day before the full moon, but wholly disappeared on the day of full moon and the following day.

In the male fluctuations of both spent and mature individuals in the period were found quite parallel to those in females. However, the spent male never reached 100% and the mature male never went down to zero throughout the period.

III. The third period

The examination of gonad was made only in three days, August 16, 17 and 18, the new moon being on the 16th. A typhoon passed through the vicinity two days before the new moon and the rough water made the collecting of sea urchins in shore impossible in these two days.

Both females and males were spent or wholly spent on the day of new moon and there was no individual in grade higher than III in both sexes. Similar condition was continued in the following two days in the female, but in the male some individuals in grade III appeared on the next day of new moon. It must be noted that some males marked with an asterisk in Table 2c began the discharge of sperms soon after they were taken out of the water and resultantly they were in the state of spent or nearly spent at the time of examination. It is impossible to guess exactly the grade of maturation in which those males had been when they were collected, although it is clear that they had been in a certain condition ready to shed sperms by slight stimulations.

Table 2b. Records of examined individuals in the second period of observation.

sex / date	female			male		
	test volume (cc)	GT ratio	maturity grade	test volume (cc)	GT ratio	maturity grade
29/VII	50.4	20.0	III	58.0	25.0	III
	34.0	22.1	III	54.5	30.3	IV
	35.0	30.0	IV	35.0	34.3	IV
	42.0	46.4	V			
	25.0	48.0	V			
	32.0	35.9	IV			
	26.5	34.0	IV			
30/VII	61.5	29.3	III	74.5	20.8	III
	39.0	34.6	IV	35.5	33.8	IV
	31.5	39.7	IV	39.0	15.4	II
	37.0	14.9	II	33.0	34.8	IV
				32.0	25.0	III
				25.0	20.0	III
				56.0	17.9	II
				50.0	9.0	I
31/VII	72.5	26.9	III	89.0	15.7	II
	72.0	10.4	II	65.0	15.4	II
	26.5	11.3	II	32.0	20.3	III
	32.5	7.7	I	34.5	33.3	IV
	43.0	9.3	I	29.5	16.9	II
	61.0	6.6	I	75.0	6.0	I
				37.5	8.0	I
1/VIII	70.5	29.1	III	110.0	25.9	III
	113.5	18.5	II	75.5	25.8	III
	100.0	4.5	I	65.0	17.7	II
	50.0	8.0	I	65.5	34.4	IV
	50.0	22.0	III	49.5	7.1	I
	36.5	8.2	I	33.0	9.1	I
	27.0	13.0	II			
	80.0	36.3	IV			
2/VIII	79.0	10.1	II	56.0	9.8	I
	70.0	7.9	I	88.0	19.3	II
	56.0	11.6	II	55.0	39.1	IV
	41.0	7.3	I	63.5	14.2	II
	33.0	13.6	II	50.1	19.8	II
	50.0	5.0	I	33.5	11.9	II
				36.0	12.5	II

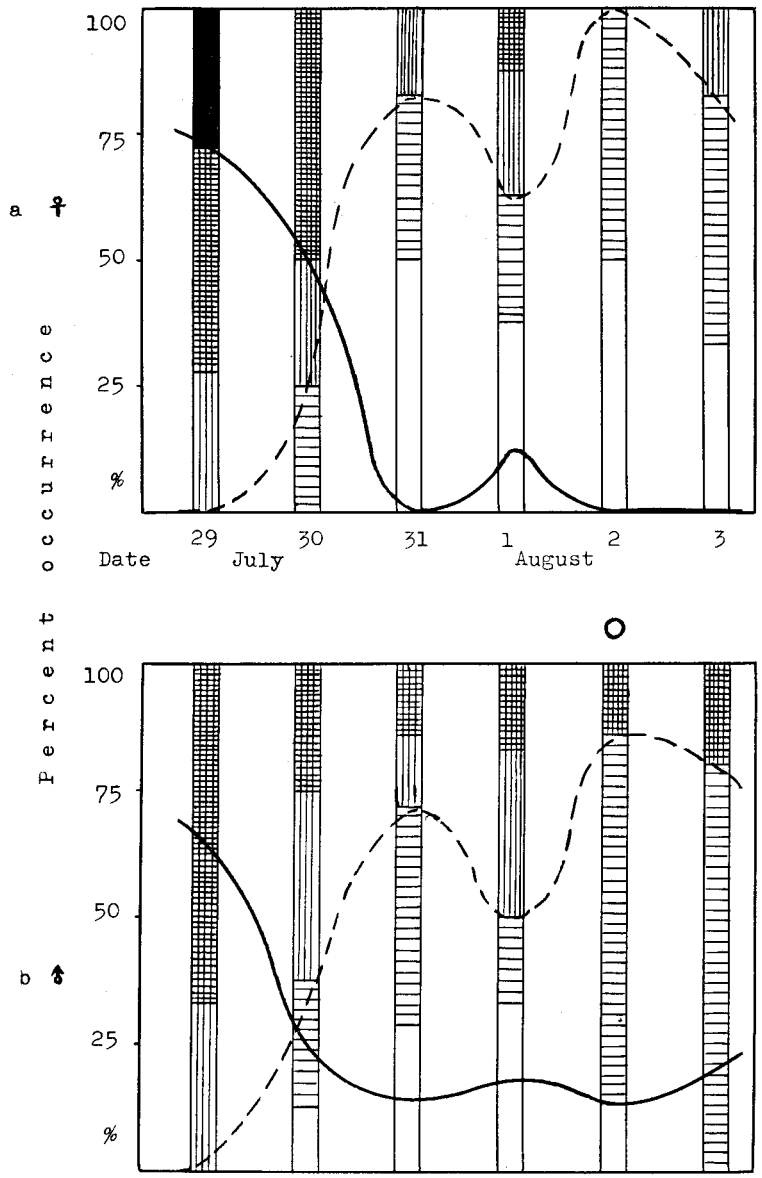
Table 2b. (continued)

sex / date	female			male		
	test volume (cc)	GT ratio	maturity grade	test volume (cc)	GT ratio	maturity grade
3/VIII	44.5	18.0	II	41.5	15.7	II
	53.0	12.3	II	38.5	31.2	IV
	53.0	9.4	I	30.5	14.8	II
	44.0	21.6	III	70.5	11.3	II
	45.0	17.8	II	53.5	18.7	II
	28.5	8.5	I			

Table 2c. Records of examined individuals in the third period of observation.

sex / date	female			male		
	test volume (cc)	GT ratio	maturity grade	test volume (cc)	GT ratio	maturity grade
16/VIII	66.5	11.3	II	37.5	12.0	II
	35.5	14.1	II	22.5	17.8	II
	37.5	10.7	II	19.5	18.9	II
	35.5	12.7	II	34.5	4.4	I
	37.0	14.8	II	34.0	10.3	II
17/VIII	57.5	13.4	II	*34.5	21.7	III
	43.5	17.2	II	*35.0	15.7	II
	46.5	17.2	II	47.5	14.8	II
	34.5	8.7	I	33.5	23.9	III
	37.5	13.3	II	33.0	10.0	II
	34.0	19.1	II			
18/VIII	54.5	10.1	II	*76.5	6.5	I
	41.5	10.9	II	*34.5	11.3	II
	31.5	11.1	II	*31.5	6.3	I
	32.0	7.8	I	21.5	11.6	II
				20.5	7.3	I
				20.0	7.5	I
				19.5	5.1	I

* These individuals began the discharge of sperms just after they were collected.



Text-figure 2. Occurrences of individuals of respective maturation grades in percent in the period from July 29 to August 3, 1966, with the full moon on the 2nd. For signs see Text-fig. 1.

Conclusions

In many kinds of sea urchins it has been reported that the reproduction is repeated in a certain rhythm correlated with the lunar cycle. In these species mature individuals in both sexes discharge genital products and become spent in a period round the full moon during the breeding season (literatures referred to in reviews by HARVEY (1956) and BOOLOOTIAN (1966) and in the first paper of this series). However, MOORE, JUTARE, BAUER and JONES (1963) suggested the possibility of spawning at the time of new moon as well as at the full moon in *Lytechinus variegatus*, and KOBAYASHI (1967) discussed on such a hemi-lunar periodicity of spawning in *Mespilia globulus* at Seto. The results obtained in the present observations with *Diadema setosum* seems to be sufficient to confirm this view. In both sexes, the percent of spent individuals inclusive of wholly spent ones attained the maximum in a few days around the full moon in the beginning of August and fluctuations in the occurrence of mature individuals were found quite in contrast with those of spent ones. These data are enough to admit the enhancement of reproductive activity in the period of full moon as has been reported in some sea urchins. In addition, the occurrence of spent females reached another mode on the day of new moon in July and evidently on both sides of this mode decreased. Changes in the occurrence of mature individuals followed quite reversely those of spent and thus attained a minimum on the next day of the new moon. In a few days ensuing the new moon in August nearly all of examined individuals in both sexes were in the state of spent or nearly spent. These data seemingly point to the possibility of spawning in the period round the new moon. Thus, the reproductive rhythm in *Diadema setosum* at Seto appears to be of a hemi-lunar periodicity as in *Mespilia*.

It is noticed that any wholly spent individual was not found in both sexes during the period of new moon in July and the same was true of *Mespilia* at Seto (KOBAYASHI 1967). According to YOSHIDA (*loc. cit.*) *Diadema setosum* at Misaki is said to reach maturity in the beginning of July. Such an insufficient evacuation of gonads as indicated by individuals examined in July is considered to be a feature characteristic to the spawning mode in the early period of the breeding season. Fluctuations in the occurrence of individuals in respective maturation grade during the period of the first observation seem to suggest that the gonad will be again full of ripe genital products in a few days after the spawning. This can be explained as follows: the bulk of germ cells do not mature at a time of the spawning, but some part remains immature and become ripe to be discharged within a few ensuing days.

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